Journal of Heredity 2005:96(2):132–144
doi:10.1093/jhered/esi020
Advance Access publication December 23, 2004

Chromosomal Assignment of RFLP Linkage Groups Harboring Important QTLs on an Intraspecific Cotton (*Gossypium hirsutum* L.) Joinmap

M. Ulloa, S. Saha, J. N. Jenkins, W. R. Meredith, Jr., J. C. McCarty, Jr., and D. M. Stelly

From the USDA-ARS, WICS, Res. Unit, Cotton Enhancement Program, Shafter, CA 93263 (Ulloa); USDA-ARS, Crop Science Research Laboratory, P.O. Box 5367, Mississippi State University, MS 39762 (Saha, Jenkins, McCarty); USDA/ARS, Crop Genetics and Production, Stoneville, MS 38776 (Meredith), and Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843 (Stelly).

Address correspondence to M. Ulloa at 17053 N. Shafter Ave., Shafter, CA 93263, or e-mail: mulloa@pw.ars.usda.gov.

Abstract

Chromosome identities were assigned to 15 linkage groups of the RFLP joinmap developed from four intraspecific cotton (*Gossypium hirsutum* L.) populations with different genetic backgrounds (Acala, Delta, and Texas Plains). The linkage groups were assigned to chromosomes by deficiency analysis of probes in the previously published joinmap, based on genomic DNA from hypoaneuploid chromosome substitution lines. These findings were integrated with QTL identification for multiple fiber and yield traits. Overall results revealed the presence of 63 QTLs on five different chromosomes of the A subgenome (chromosomes-03, −07, −09, −10, and −12) and 29 QTLs on the three different D subgenome (chromosomes-14 Lo, −20, and the long arm of −26). Linkage group-1 (chromosome-03) harbored 26 QTLs, covering 117 cM with 54 RFLP loci. Linkage group-2, (the long arm of chromosome-26) harbored 19 QTLs, covering 77.6 cM with 27 RFLP loci. Approximately 49% of the putative 92 QTLs for agronomic and fiber quality traits were placed on the above two major joinmap linkage groups, which correspond to just two different chromosomes, indicating that cotton chromosomes may have islands of high and low meiotic recombination like some other eukaryotic organisms. In addition, it reveals highly recombined and putative gene abundant regions in the cotton genome. QTLs for fiber quality traits in certain regions are located between two RFLP markers with an average of less than one cM (0.4∼0.6 Mb) and possibly represent targets for map-based cloning. Identification of chromosomal location of RFLP markers common to different intra- and interspecific-populations will facilitate development of portable framework markers, as well as genetic and physical mapping of the cotton genome.

Introduction

The demands for food and fiber justify the need for innovative approaches to identify and manipulate genes responsible for economically important quantitative trait loci (QTL). The genetic dissection of complex traits into QTLs renders individual loci amenable to analysis like single Mendelian factors and to individual or collective manipulation through marker-assisted selection. The definition of linked molecular markers is essential to the detection and marker-assisted selection of complex traits. It is important that such QTLs and markers be defined in cotton, the world's most important textile fiber crop and one of the most important sources of oil seeds (Percival et al. 1999; Wendel et al. 1992).

DNA markers linked to agronomic traits increase the efficiency of breeding and significantly decrease the cost, time, and risk of subjective phenotypic assays. The two major limiting factors in the use of molecular markers for both QTL analysis and marker-assisted selection programs in cotton are as follows: (1) the limited number of suitable markers available in the public sector and (2) the lack of knowledge of how these markers are associated with economically important QTLs. The genetic map of RFLP markers and QTLs in defined chromosomal regions will be very helpful in transferring useful orthologous QTL loci among the cotton germplasm. Cytogenetic analyses indicated that cultivated cotton (*G. hirsutum* L.) is of polyploid origin and that, by virtue of the complementation between its two

compensating homoeologous genomes, cotton generally tolerates deletions and deficiencies at the single or partial chromosome level. Currently, a large number of interspecific aneuploid chromosomal substitution stocks are available in cotton. The comparative analysis of the interspecific chromosomal substitution lines of tetraploid cotton provides an opportunity in developing a chromosome-based consensus map of important traits and molecular markers within a very short period (Endrizzi et al. 1985; Saha and Stelly 1994). A chromosome-specific molecular mapping strategy will also serve as an anchor for integrating large genome segments originating from megabase technologies like the BAC (Tomkins et al. 2001). This approach will also be a key tool for map-based chromosome walking and cloning of desirable genes. In addition, the identification of chromosomal regions with economically important traits will be especially important in germplasm improvement and introgression programs using interspecific chromosome substitution lines (Saha et al. 2003). Economic importance is underscored by the fact that Upland cotton (*G. hirsutum*) occupies 95% of U.S cotton production on account of its higher yield and wider adaptation. However, exceptional fiber strength, fineness, and superior spinning and manufacturing performance give Pima and Sea Island cotton types (*G. barbadense*) a 30%–50% price advantage over Upland cotton.

Recent advances in molecular genetics offer a rapid and precise approach to plant improvement through the identification and characterization of genes controlling traits. RFLP genetic linkage maps have been employed to further advance genetic knowledge in other crops (Tanksley et al. 1992; Xiao et al. 1996). Several reports provided valuable information on RFLP markers linked to important QTLs using different inter- and intraspecific populations in cotton (Reinisch et al. 1994; Shappley et al. 1998; Jiang et al. 2000; Yu et al. 1998; Ulloa et al. 2000; Ulloa and Meredith 2000; Ulloa and Meredith 2002; Paterson et al. 2003). Recently, scientists from different countries initiated the International Cotton Genome Initiative (ICGI) to coordinate future cotton genomics research (http://www.jcotsci.org/2000/issue02/toc.html). ICGI stressed the need for more portable and publicly available framework markers to expedite cotton genomic research. Integrating map information from different populations to develop a consensus map based on common RFLP loci has several advantages over that based on a single population, including the detection of a larger number of mapped loci, better estimation of the gene order and map distances, the identification of possible chromosomal rearrangements, and the detection of consensus markers (Kianian and Quiros 1992; Austin and Lee 1996; Cregan et al. 1999; Ulloa et al. 2002). Recently, Ulloa et al. (2002) presented such a report on the first cotton genetic linkage joinmap assembled in *G. hirsutum* with a core of common RFLP markers assayed on different cotton populations (Acala, Delta, and Texas Plains). The overall objective of this paper is to identify the chromosomal location of important QTLs linked to RFLP markers in cotton using aneuploid chromosomal substitution lines (F_1), common probes between different populations used in the joinmap, and QTL linkage map reports (Shappley et al. 1998; Ulloa and Meredith 2000; Ulloa et al. 2002).

In this report, we augment previously published information in the following manner: (1) we associate a number of molecular markers and their linked QTLs to specific chromosomes; (2) we used cDNA as marker probes so that the marker data eventually can provide information about functional genes; (3) the results will demonstrate the portability and strength of the RFLP markers in the genetic analysis of cotton between different intra- and interspecific populations; (4) these RFLP markers can be used as framework markers specific to different chromosomes in cotton; and (5) present results that suggest there are islands of high and low meiotic recombination and gene-rich regions in cotton from *G. hirsutum*×*G. hirsutum* crosses.

Materials and Methods

Mapping Populations Used for the Joinmap Construction

In the first mapping population (Pop 1) (Shappley et al. 1998), the breeding line MARCABUCAG8US-1–88 (MAR) and the cultivar "HS46" were chosen as parental lines. Two agronomically and geographically divergent cultivars (MD5678ne and "Prema") were used to develop the second mapping population (Pop 2) (Ulloa and Meredith 2000). A third set of agronomically and geographically distinct parent cultivars (HQ95–6 and "MD51ne") were used as parents to create population 3 (Pop 3) (Ulloa et al. 2002). The fourth population (Pop 4) was developed from two parents ("DES 119–5" and MD51ne) with very dissimilar phenotypes but a common geographic origin (Ulloa et al. 2002).

Probe Construction and RFLP Analysis

A cDNA library using the pGem-11zt(-) vector (Promega) was developed from the leaf tissue (***C***) and fiber tissue (***F***) of six cotton cultivars, from which a set of 376 probes was generated at Biogenetic Service, Inc., of Brookings, South Dakota (BGS) and used in this study. Information concerning the availability of the RFLP probes used in this study may be obtained directly from Biogenetic Services, Inc.

RFLP methods described by Ausubel (1978) were used with some minor modifications by Biogenetic Services, Inc. (unpublished). Either EcoRI (I at end of named locus) or EcoRV (V at end of named locus) restriction enzymes were used in the digestion of the DNA sample, depending on the probe employed. A total of 376 probe/enzyme combinations were used to assemble the joinmap using the RFLP information from the populations (Ulloa et al. 2002).

RFLP analyses were conducted using DNA from bulk samples of juvenile leaf tissue from individual $F_{2:3}$ family single row-plots. Each row-plot had at least 30 plants. Pop 1 was assayed using 129 probe/enzyme (*Eco*RI and EcoRV) combinations (Shappley et al. 1998). Pop 2 was assayed using 106 probe/enzyme (EcoRI and EcoRV) combinations (Ulloa and Meredith 2000). Pop 3 was assayed using 94 probe/enzyme (EcoRI and EcoRV) combinations. A codominant

pollen color marker also was scored in this population (Ulloa et al. 2002). Pop 4 was assayed using 63 probe/enzyme (EcoRI and EcoRV) combinations (Ulloa et al. 2002).

Chromosome Substitution Stocks

The probes and restriction enzyme combination were selected from the RFLP linkage groups with putative QTLs from genetic maps developed by Shappley et al. (1998) and Ulloa and Meredith (2000).

The DNA isolated from the leaves of chromosome (Chr) substitution stocks, their recurrent parents' common ancestor (inbred *G. hirsutum* Texas Marker-1 (TM1)), and their common *G. barbadense* donor (doubled haploid line (3–79)) were used as standards to compare with DNA from leaves of euploid F_1 (2n=52), monosomic F_1, and monotelodisomic F_1 chromosome substitution plants that were developed from interspecific crosses between *G. hirsutum* hypoaneuploids and 3–79. The *G. hirsutum* hypoaneuploids' parents were quasi-isolines of TM1, derived by various numbers of backcrosses to TM1. The RFLPs were assigned to cotton chromosomes and chromosome arms in a manner described by Stelly (1993) and Liu et al. (2000). By virtue of the absence in each hybrid for a specific *G. hirsutum* chromosome, the interspecific hybrid stocks were monosomic for *G. barbadense* chromosomes 1, 2, 3, 4, 6, 7, 9, 10, 12, 16, 18, 20, and 25. These enabled assignment of RFLP markers to entire chromosomes. In addition, stocks monotelodisomic (Te) for *G. barbadense* chromosome arms 5Lo, 12Lo, 14Lo, 15Lo, 22Lo, 25Lo, 17sh, or 26sh were used in our RFLP project. The stocks were obtained from the Cotton Cytogenetics Collection at Texas A&M University (vegetative duplicates of which are maintained at the USDA-ARS at Mississippi State University). The collection of hypoaneuploids lacking a single chromosome or single chromosome arms was developed by testing aneuploids with translocations to determine the identity of the missing chromatin (Stelly 1993). The stocks are labeled for the missing *G. hirsutum* (TM1) chromosome. For example, H 3 Sub F_1 indicates that the *G. hirsutum* chromosome 3 is lacking. On the other hand, the monotelodisomic stocks are labeled by the particular chromosome arm that is present. Thus, Te 5 Lo Sub F_1 denotes that only the long arm of the *G. hirsutum* chromosome 5 is present, but the short arm of the *G. hirsutum* chromosome 5 is missing. TM1 is an inbred line that is considered as the genetic standard of Upland Cotton (Kohel 1973), and 3–79 is a doubled haploid (i.e., it is also highly homozygous and can be considered as a cytogenetic standard for the *G. barbadense* cotton types). The monosomic stocks are interspecific F_1 substitution stocks where a particular *G. hirsutum* chromosome is missing although the homologous *G. barbadense* chromosome is present. All of these hypoaneuploid lines, each lacking a specific TM1 chromosome or chromosome arm, were cytologically identified to determine the nature of the missing chromatin.

Chromosome Assignment

The overall strategy of identifying chromosomal locations followed the method of monosomic F_1 deletion analysis (Saha and Stelly 1994; Liu et al. 2000). The authors assigned loci to chromosomes (Chr) and chromosome arms (sh=short arm; Lo=long arm) as per the designation of monosomic and monotelodisomic stocks described by Stelly (1993). To localize codominant RFLP loci to chromosomes, we screened chromosome-deficient interspecific hybrid stocks for hemizygosity versus heterozygosity at RFLP loci. In each hypoaneuploid F1, disomic segments retain natural F1 heterozygosity for the parental alleles, whereas all RFLP loci in the missing *G. hirsutum* segment lack the maternal allele and are therefore hemizygous for the paternal 3–79 allele. Dominant molecular markers carried by the maternal *G. hirsutum* cytogenetic stock parent were localized similarly (i.e., by absence of the allele in hypoaneuploid F_1 hybrid stocks with a specific *G. hirsutum* chromosomal or segmental deficiency).

Genetic loci detected by RFLP markers have been designated by the name of the DNA probe and restriction enzyme combination. If a probe detected RFLPs at more than one locus, letters (a, b, c) were arbitrarily assigned alphabetically from higher size to lower size (e.g., 2kb RFLP of the same probe as a versus 600bp RFLP as b). The allelic nature of each RFLP locus was determined according to principles described by Reinisch et al. (1994). We selected at least two probes from each of the RFLP linkage groups (G) of Shappley et al. (1998) to identify and confirm the chromosomal location of the linkage group (Table 1). Surprisingly, many of the polymorphic alleles selected from the linkage groups of Shappley et al. (1998) exhibited monomorphic banding patterns between the parents of the chromosome substitution lines; accordingly, they could not be used for the deletion mapping strategy to identify chromosomal location. For some of the affected linkage groups, only one polymorphic locus was available for chromosomal identification, which we therefore consider tentative until corroborating data are accumulated.

Genetic Linkage Analysis and Map Construction

Details of the RFLP analysis, linkage analysis, and construction of the genetic linkage maps are presented in Ulloa et al. (2002). The JoinMap^R (Stam and Van Ooijen 1995) software was used to test for Chi-square goodness-of-fit for expected versus observed genotypic ratios and to develop the final genetic maps. Mapmaker/Exp 3.0 (Lander et al. 1987) and JoinMap^R (Stam and Van Ooijen 1995) were used to develop these genetic maps. LOD scores of 3–6 were examined, using the Kosambi mapping function, and a maximum distance of 40 cM was used to determine linkage between two markers. The JoinMap^R (Stam and Van Ooijen 1995) program was used to assemble the four maps, using the modules JMREC for recombination estimation; JMHET for heterogeneity testing; JMPWG for linkage group assignment and for merging linkage data obtained from the above four populations; and, finally, JMMAP for map construction. The cotton genetic joinmap presented herein uses the same RFLP data set and the same four cotton mapping populations from Ulloa et al. (2002).

Table 1. Selected cDNA probes from the RFLP linkage groups to identify and confirm the chromosomal location of the linkage group in this study. Asterisk represents cDNA probe/RFLP as signed to a chromosome.

Joinmap Linkage Group	Shappley Linkage Group	cDNA probes/RFLPs	Chromosome (Chr)	Joinmap Linkage Group	Shappley Linkage Group	cDNA probes/RFLPs	Chromosome (Chr)
	3	C45F1I			**20**	C28C1V	
		C81A5I				F3F7I	
1	**4**	C46F1I		**43**	**21**	C33A6I	
		C34E3V*	Chr 03			F6D4V*	Chr 10
		C108C5V					
		C111A6I					
11	**5**	C56D6V			**23**	C44D2I	
		F12D4V*	Chr 05			C87E4I	
7	**6**	C56E1V/aI			**24**	C113C4I	
		C84B3I*	Chr 07			C114B1I	
						C792aI	
	7	C15A4V		**45**	**25**	C101D1V	
		C50D1V				C106D2V*	Chr 14
		C45B6V					
	9	C81F4aI			**27**	C116E4I/V	
		C61A1aI					
2	**10**	C61A1bI			**28**	C88C2I	
		C116D3V				C117B3I	
		C112E6V				C118C3I	
		C89B4V					
		C81F4bI					
		C34F5V*	Chr 26				
6	**11**	F3B9V		**20**	**29**	C41E4I	
		C65C2I*	Chr 20			C119F6I*	Chr 03
		C43E4V					
		C19B3V					
42	**12**	C17A6I		**2**	**30**	C117F1I*	Chr 26
		C78C3I*	Chr 12			C61A6V*	
	13	C38E2I		**13**	**31**	C103B1V	
		C87F1V				C13B1V*	Chr 26
		C112F3I					
1	**14**	F2E6I*	Chr 03				
		F4B4V					
		C104A1I					
		C100A2V					
		C117C5V*					
14	**15**	C52E2V					
		C107B2a/bI*	Chr 26				
3	**16**	C13F3V*	Chr 03				
		C16F4aV					
26	**17**	F2C11V*	Chr 09				
		C17F2V					
		C86B3I					
		F3B2I					
36	**18**	C18A4I/V*	Chr 03				
12	**19**	C80F1V					
		C56A6aI					
		C27B6I					
		F9D3V					
		F2A4V*	Chr 03				

Agronomic and Fiber Quality Data

The agronomic and fiber quality data from the 96 $F_{2:3}$ families of Pop 1 were obtained from the publication of Shappley et al. (1998). These data were collected at one site in a two-row replicated test in 1995. The 119 $F_{2:3}$ families from Pop 2 (Ulloa and Meredith 2000) were grown in one-row plots at two sites in 1991. The plot size was 1 m wide and 5 m long. Tests were planted in two-replicate, randomized complete block designs. The data collected included yield, yield components, and fiber properties. Plant density was about 113,000 plants ha^{-1}. Weed control, irrigation, and insect control were standard practices for production of cotton in the Mississippi Delta. Boll

weight was determined from 50 hand-harvested bolls, just prior to the first harvest from each plot. Lint percentage was determined from the 50-boll sample by ginning on a small, 10-saw experimental gin. The agronomic traits evaluated were lint yield, lint percentage (Lint% and lp), boll weight, seed weight, seed index (sdx), and nodes.

Fiber properties were determined by Starlab, Inc., of Knoxville, Tennessee. The fiber quality traits evaluated included fiber span length at 50% (50% SL) and at 2.5% (2.5% SL), fiber bundle strength in kN m kg^{-1} (t1), fiber elongation (e1), micronaire reading (mic), fiber maturity (mat), fiber perimeter (per), and arealometer instrument measures: a, ah, weight fineness (wfns), and so forth. Additional information can be obtained from the published manuscripts for Pop 1 by Shappley et al. (1998) and for Pop 2 by Ulloa and Meredith (2000).

Quantitative Trait Loci (QTL) Analysis Programs

For Pop 1, the mixed model approach was used to search for QTLs along the linkage groups by a step of 2.0 cM. In the mixed model approach, effects of QTLs are considered fixed; effects of molecular markers are random. A likelihood ratio value threshold (LR) of 7.88 or above was chosen, which provided significance with a probability of 0.001, with one degree of freedom (Shappley et al. 1998).

QTL analyses of Pop 2 (Ulloa and Meredith 2000) were performed with three different computer programs: Mapmaker/QTL (Lander et al. 1987), MapQTL (van Ooijen and Maliepaard 1996), and QTL Cartographer (Basten et al. 1994). Using the permutation test (Churchill and Doerge 1994) provided as a part of QTL Cartographer Software, and 1,000 permutations for each test, the criterion for QTL significance for all of the above traits ranged from 6.50 to 13.5 likelihood ratio test statistic (LR). The threshold value of 9.21 for the LR was set for declaring a QTL for previously reported QTLs for Pop 2 (Ulloa and Meredith 2000).

Results

Tests were conducted to identify the chromosomal locations of 74 genetic loci detected by RFLP markers described by Shappley et al. (1998). Of the 74 loci, 19 RFLP loci were assigned to nine cotton chromosomes, but 12 of the polymorphic loci were associated with just two chromosomes. Seven RFLP loci were associated with chromosome 3, and five RFLP loci were associated with chromosome 26 (Table 1). We used more than one probe per linkage group for most linkage groups. Approximately 15% of the probes that revealed polymorphism in the intraspecific mapping population of Shappley et al. (1998) failed to detect polymorphism between TM-1 and 3–79 and therefore could not be localized by the tests reported here. Loci detected as codominant or dominant TM-1 RFLP alleles were assigned to a chromosome by monosomic F_1 deletion analysis (Saha and Stelly 1994; Liu et al. 2000; see Figure 1). Loci detected as dominant 3–79 alleles could not be localized based on the deletion analysis because all of the aneuploid F_1 plants carried a full haploid complement of paternal chromosomes (3–79). RFLP loci for which scoring was difficult were not used to assign chromosomes in this study. Only RFLP loci for which TM-1 carries a dominant or codominant allele were used to assign a chromosome (Figure 1). Additional research is needed to confirm further the precise chromosomal placement for the linkage groups below.

Chromosomal Location of Different RFLP Loci to Linkage Groups of Ulloa et al. (2002)

The joinmap included 283 loci plus one morphological marker (P_1) (making a total of 284 loci) that were found to be linked with an LOD of 4.0, covering 1,502.6 cM, based on the JoinMap^R computer program (Stam and Van Ooijen 1995). The 284 loci were mapped to 47 linkage groups and covered approximately 31% of the total recombinational length of the cotton genome with an average distance between two markers of 5.2 cM (Ulloa et al. 2002). Fifteen linkage groups from the above joinmap were assigned to specific physical cotton chromosomes, covering 769.3 cM and discussed herein.

In Figures 2, 3 and 4, we present our results based on the polymorphic RFLP loci and individual chromosome as follows.

Chromosome 3. Our results showed that chromosome 3 was associated with five different linkage groups of the joinmap (Figure 2). The joinmap linkage group 1 was assigned to chromosome 3 based on F2E6I, C117C5V (Pop 1 G-14), and C34E3V (Pop 1 G-4) polymorphic loci. TM-1-specific alleles were present in all of the aneuploid substitution lines except the monosomic plant deficient for chromosome 3, indicating association of this linkage group with this chromosome. These results agreed with a previous report (Saha et al. 2000), in which F2E6I and C117C5V probes were localized to chromosome 3. TM-1 alleles for solitary markers of several other linkage groups were also deficient from the chromosome 3 hypoaneuploid. The joinmap linkage group 3 was tentatively assigned to chromosome 3 based on results with the C13F3V (Pop 1 G-16) polymorphic probe. The TM-1-specific allele was present in all of the aneuploid substitution lines except the monosomic plant deficient for chromosome 3, indicating association of this linkage group with this chromosome. This was the only locus of the joinmap linkage group 3 that could be detected with the set of selected probes. The monosomic F_1 plant missing TM-1 chromosome 3 also lacked the TM-1 allele of F2A4V (Pop 1 G-19) RFLP locus of the joinmap linkage group 12, the C119F6I (Pop 1 G-29) TM-1 allele of the joinmap linkage group 20, and the C18A4V (Pop 1 G-18) TM-1 allele of the small joinmap linkage group 36 (Figure 2), leading to their tentative association with Chr-03.

Short Arm of Chromosome 5. The joinmap linkage group 11 was assigned to the short arm of chromosome 5 based on the F12D4V (Pop 1 G-5) polymorphic probe. The TM-1-specific allele was present in all of the aneuploid substitution lines except the monosomic plant deficient for chromosome 5Lo, indicating association of this linkage group with the short arm of chromosome 5. We also observed that the locus for pollen color (P_1), which resides in

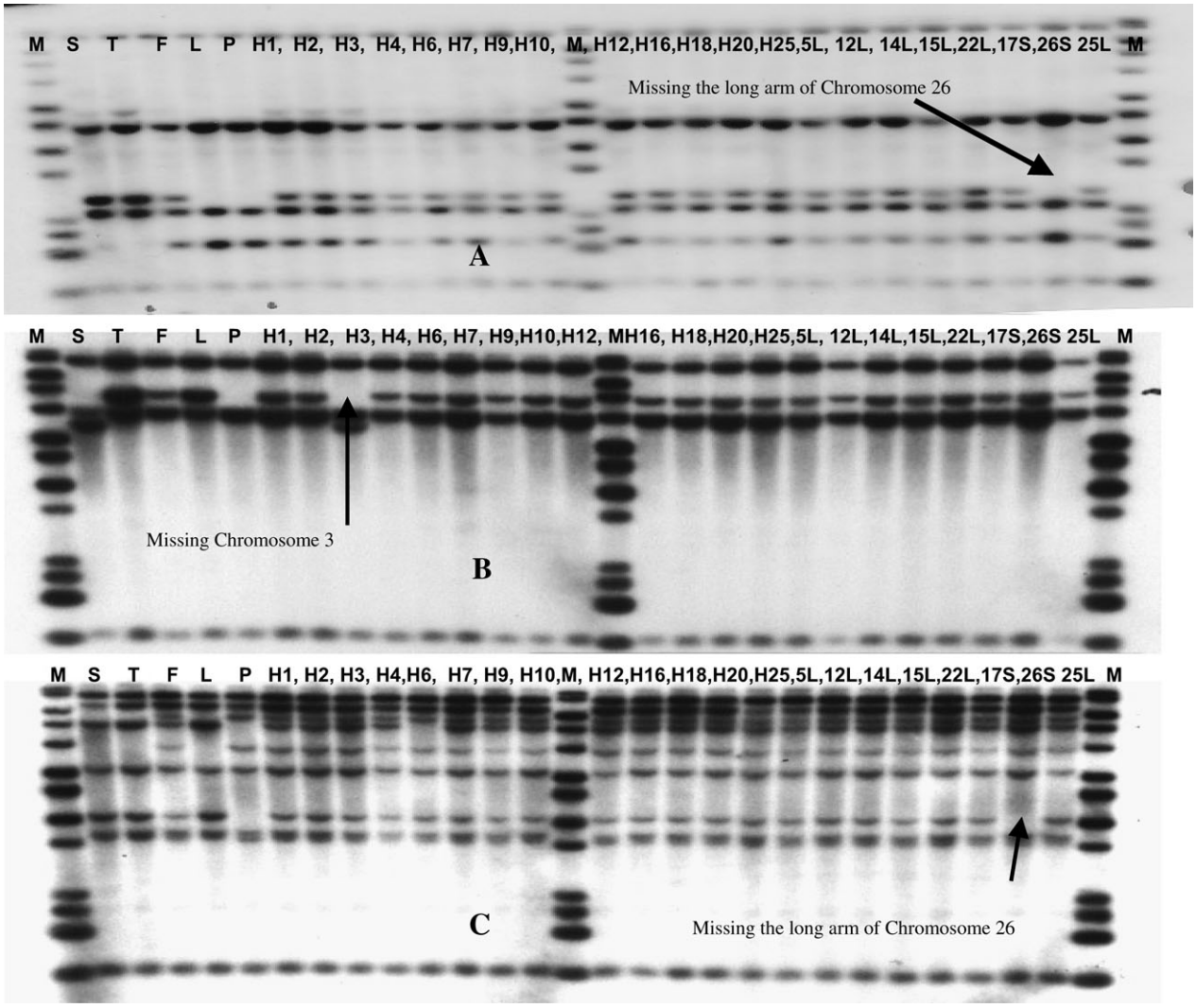

Figure 1. Images of X-ray films showing different cDNA probes used to assign cotton chromosomes to specific linkage groups. (A) Probe C107B2 with *Eco*RI; (B) Probe F24V with *Eco*RV; and (C) C117F1 with *Eco*RI. M=molecular standard, S=Upland cotton, T=TM1, F=F1 hybrid, L=cotton mutant, P=3–79, and H1 to 25L=monosomic F_1 and monotelodisomic F_1 chromosome substitution plants developed from interspecific crosses between TM1 and 3–79. For example, H 3 Sub F_1 indicates that the *G. hirsutum* chromosome 3 is lacking. Conversely, the monotelodisomic stocks are labeled by the particular chromosome arm that is present. Thus, Te 5 Lo Sub F_1 denotes that only the long arm of the *G. hirsutum* chromosome 5 is present, but the short arm of the *G. hirsutum* chromosome 5 is missing.

chromosome 5 (Endrizzi and Ramsay 1979) was also linked to the above loci (Figure 3).

Chromosome 7. The TM-1 alleles of the C84B3I (Pop 1 G-6) RFLP locus in the joinmap linkage group 7 was missing in the monosomic F_1 plant deficient for chromosome 7, indicating the presence of this locus in this chromosome (Figure 3).

Chromosome 9. The polymorphic codominant F2C11V (Pop 1 G-17) RFLP locus, TM-1-specific allele, of the joinmap linkage group 26 was present in all of the aneuploid substitution lines except the monosomic plant deficient for Chr-09 (Figure 3).

Chromosome 10. The F6D4V (Pop 1 G-21) locus of the joinmap linkage group 43 was codominant polymorphic similar to Shappley et al. (1998). The TM-1-specific allele for this locus was present in all of the aneuploid F_1 plants except the monosomic F_1 plants missing for the chromosome 10, indicating its location in this chromosome (Figure 3).

Chromosome 12. The polymorphic C78C3I (Pop 1 G-12) RFLP locus, TM-1-specific allele, of the joinmap linkage group 42 was present in all of the aneuploid substitution lines except the monosomic plant deficient for chromosome 12, indicating association of this linkage group with chromosome 12. The C17A6I locus is in the same group, but it was

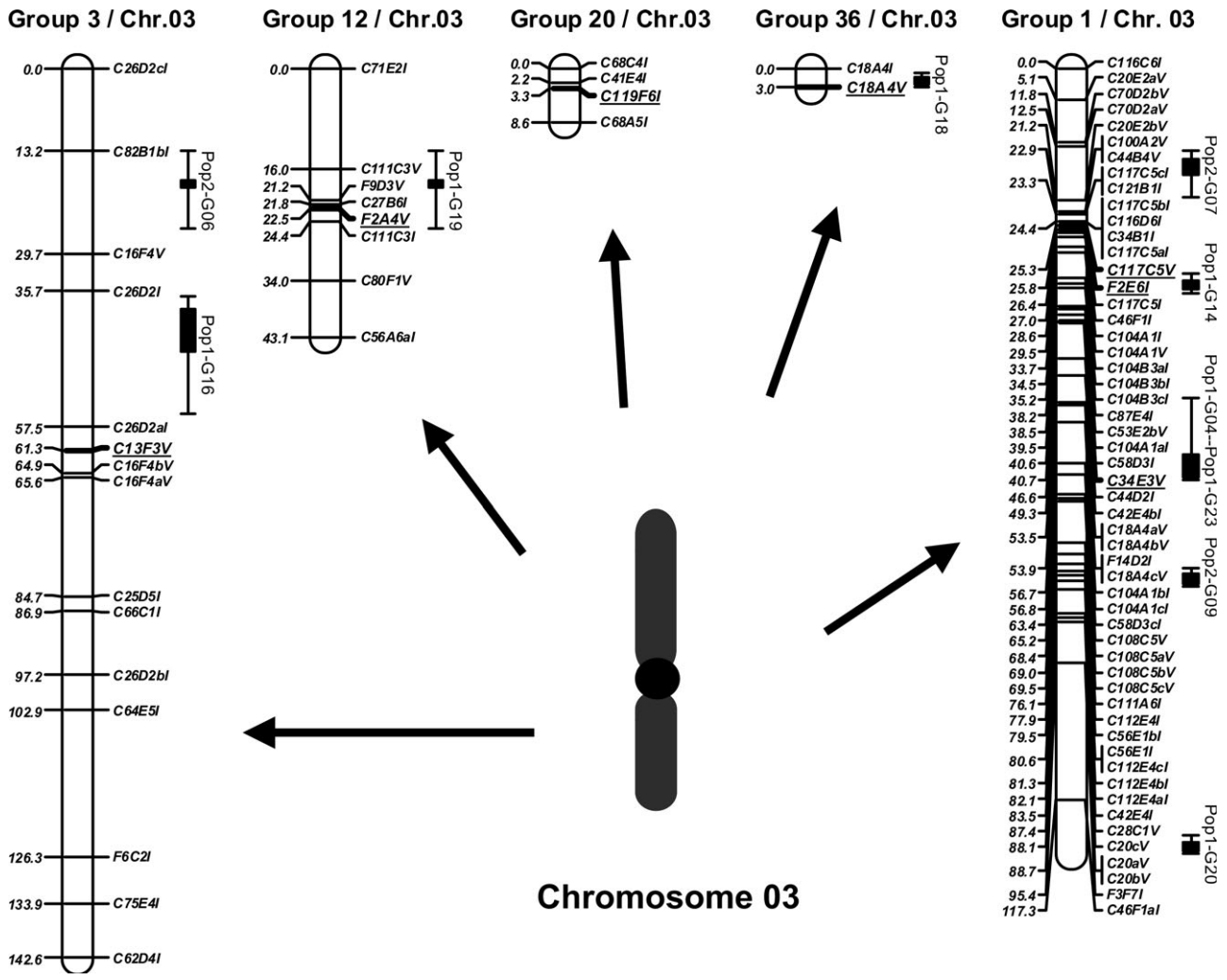

Figure 2. Joinmap linkage groups. Map distances between adjacent markers are in cM. The map was constructed using the JOINMAP (Stam and Van Ooijen 1995) computer program, with Kosambi function and LOD 4.0 (Ulloa et al. 2002). Pop 1=mapping population No 1 (Shappley et al. 1998). Pop 2=mapping population No 2 (Ulloa and Meredith 2000). Chr=chromosome, Lo=long arm of the chromosome, sh=short arm of the chromosome, G, and Group=linkage group, underlined loci on linkage groups=RFLP locus assigned to specific missing chromosome.

monomorphic between TM-1 and 3–79 and thus could not be used to detect chromosomal location (Figure 3).

Short Arm of Chromosome 14. Our results indicated that the C106D2V (Pop 1 G-25) locus from the TM1 allele of the joinmap linkage group 45 was present in all of the aneuploid F_1 plants except the monotelodisomic F_1 plant, which was missing the chromosome 14Lo. These findings indicate that the respective linkage group is associated with the short arm of Chr-14 (Figure 3).

Chromosome 20. The TM-1-specific allele of polymorphic C65C2I (Pop 1 G-11) of the joinmap linkage group 6 was present in all of the aneuploid substitution lines except the monosomic plant deficient for chromosome 20, indicating that this linkage group is associated with chromosome 20. Our results also revealed that the F3B9V, C43E4V, and C19B3V loci in the same group were monomorphic between TM-1 and 3–79 and could not be used to detect chromosomal location (Figure 3).

Long Arm of Chromosome 26. The aneuploid substitution line deficient for the long arm of chromosome 26 of TM-1 lacked TM-1 markers associated with three different linkage groups of the joinmap (Figure 4). The joinmap linkage group 2 was assigned to the long arm of chromosome 26 based on results with three polymorphic probes: C117F1I, C61A6V (Pop 1 G-30), and C34F5V (Pop 1 G-10). The TM-1-specific alleles were present in all of the aneuploid substitution lines except the monotelodisomic plant for chromosome 26sh, indicating association of this linkage group with the long arm of chromosome 26. We attempted to use two probes to detect the chromosomal locations for the joinmap of linkage group 13, namely C103B1V and C13B1V (Pop 1 G-31), but only one of them

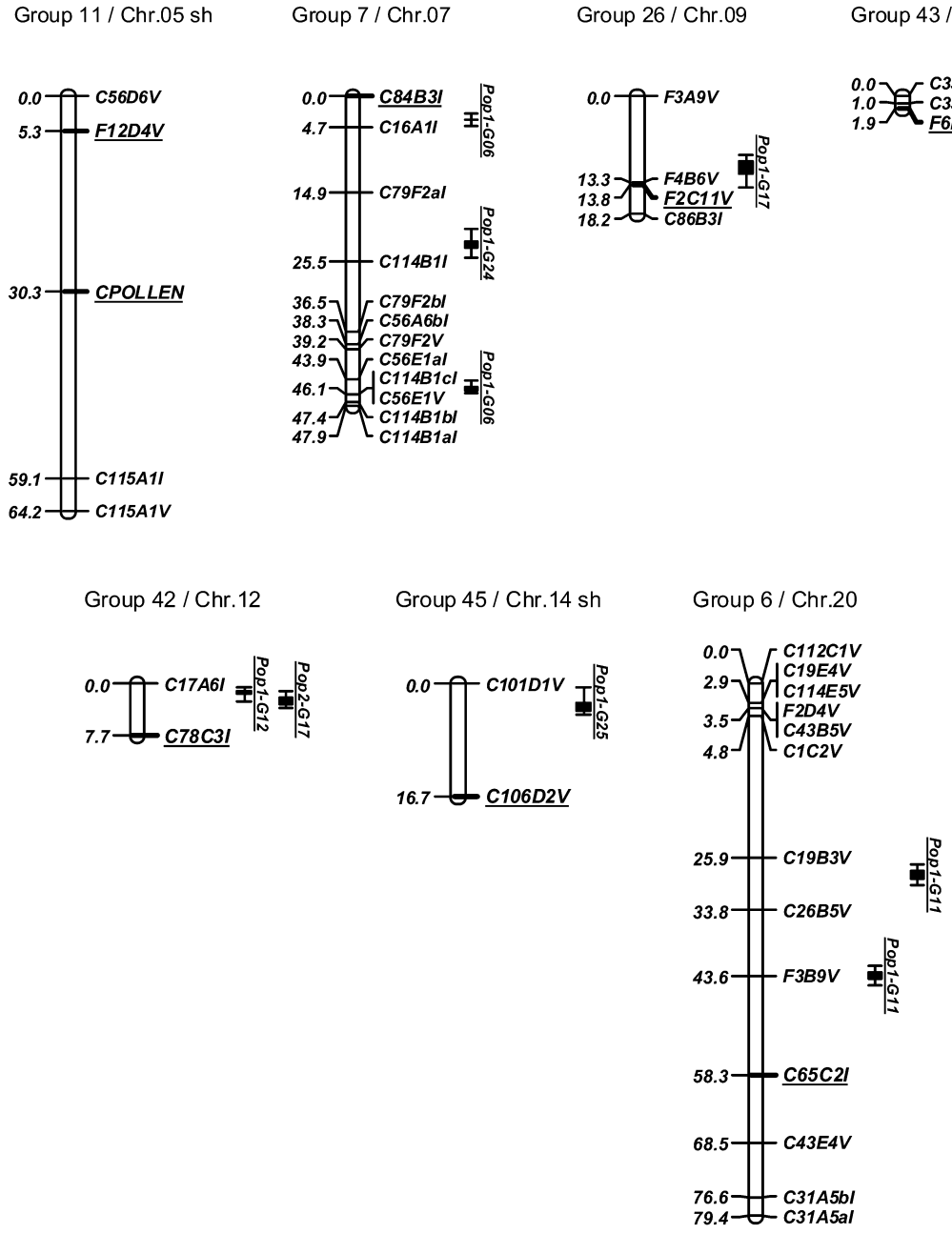

Figure 3. Cotton genetic RFLP linkage groups from the joinmap developed from four bulk-sampled plots of an $F_{2:3}$ *G. hirsutum* of four populations. Map distances between adjacent markers are in cM. The map was constructed by using the JOINMAP (Stam and Van Ooijen 1995) computer program, with Kosambi function and LOD 4.0 (Ulloa et al. 2002). The location of QTLs on a single population for agronomic and fiber quality traits was previously published. Pop 1=mapping population No 1 (Shappley et al. 1998). Pop 2 =mapping population No 2 (Ulloa and Meredith 2000). Chr=chromosome, Lo=long arm of the chromosome, sh=short arm of the chromosome, G, and Group=linkage group, underlined loci on linkage groups=RFLP locus assigned to specific missing chromosome.

was useful. C103B1V could not be mapped in the hypoaneuploid tests because its dominant RFLP allele was present in 3–79 and thus was not detectable by loss of *G. hirsutum* chromosomes. In contrast, the C13B1V RFLP marker was polymorphic between 3–79 and TM1 and thus was highly amenable to mapping. We observed heterozygosity among all of the monosomic and monotelodisomic F_1 plants except the monotelodisomic F_1 plants carrying telosome chromosome 26sh, which was hemizygous for only the 3–79 allele. Absence of the TM-1 allele indicated that the C13B1V RFLP locus is located in the long arm of chromosome 26. Analogous results were observed for the C107B2aI (Pop 1 G-15)

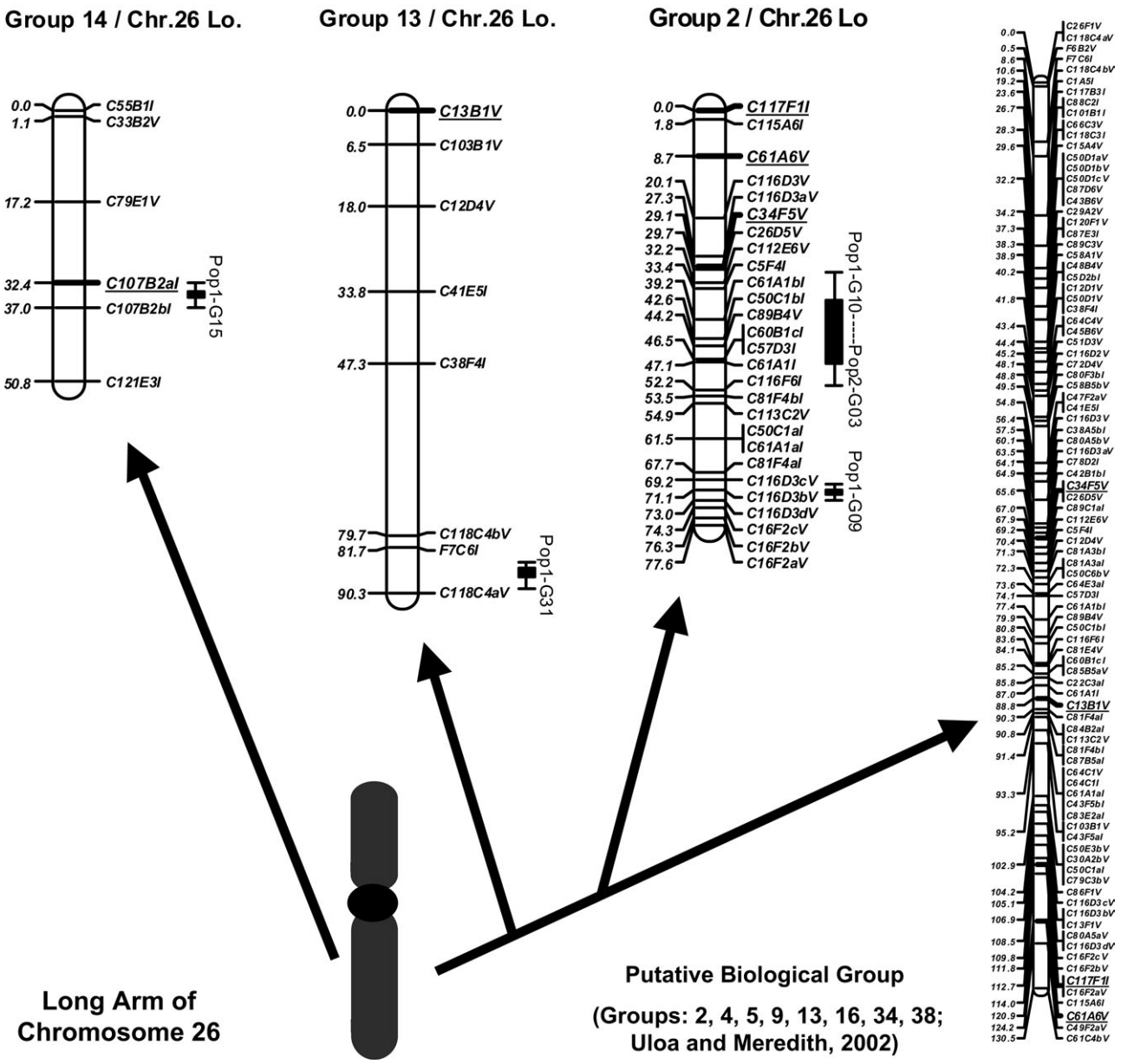

Figure 4. Joinmap linkage groups. Map distances between adjacent markers are in centiMorgans (cM). The map was constructed by using the JOINMAP (Stam and Van Ooijen 1995) computer program, with Kosambi function and LOD 4.0 (Ulloa et al. 2002). Pop 1=mapping population No 1 (Shappley et al. 1998). Pop 2=mapping population No 2 (Ulloa and Meredith 2000). Chr=chromosome, Lo=long arm of the chromosome, sh=short arm of the chromosome, G, and Group=linkage group, underlined loci on linkage groups=RFLP locus assigned to specific missing chromosome.

RFLP marker from the joinmap of linkage group 14, which indicated that the C107B2aI RFLP locus is located in the long arm of chromosome 26. Further research is needed to confirm the precise cotton chromosomal placement for the above linkage groups.

Loci and QTL Placement on the Genetic Joinmap

Based on 111 shared RFLP loci from the four populations (Pop 1, Pop 2, Pop 3, and Pop 4), the percentage of common heterozygous loci between populations varied from 9%–41% (Ulloa et al. 2002). Ninety-two QTLs from two populations were located on 15 linkage groups of the joinmap (Table 2). QTLs detected from a single population were placed on the linkage group of the joinmap (Figures 2–4) (Shappley et al. 1998; Ulloa and Meredith 2000).

Approximately 49% of the putative 92 QTLs for agronomic and fiber quality traits from the two populations were placed in two major linkage groups. Linkage group 1 (Chr-03) harbored 26 QTLs (six QTLs for agronomic traits

Table 2. Summary of reported numbers of QTLs localized to joinmap groups (Ulloa et al. 2002) based on identification in either of two intraspecific mapping populations. The QTLs from the two cotton (*Gossypium hirsutum* L.) mapping populations were divided into two trait categories (Agronomic and Fiber Quality) and detected with a likelihood ratio value≥7.88 (P≥0.005).

						Populations			
						Pop 1		Pop 2	
Trait	Genome	Cotton Chromosome	Joinmap Group	Loci No. On Joinmap Group	Joinmap Group Length (cM)	96 FamiliesHS46×MAR† *Group QTLs Detected*		119 FamiliesMD5678×Prema‡ Group QTLs Detected	
Agronomic	A	03	1	54	117.3	4	2	-	-
	A	03	3	15	142.6	14	3	-	-
	A	07	7	12	47.9	23	1	-	-
	A	12	42	2	7.7	16	1	-	-
						6	1	-	-
						-	-	17	1
	D	14 Lo	45	2	16.7	25	1	-	-
	D	20	6	13	79.4	11	1	-	-
	D	26 sh	2	27	77.6	10	2	3	1
	D	26 sh	13	8	90.3	31	1	-	-
	D	26 sh	14	6	50.6	15	1	-	-
Fiber Quality	A	03	1	54	117.3	4	1	-	-
	A	03	3	15	142.6	14	12	7	2
	A	03	12	8	43.1	20	2	9	1
	A	03	36	2	3.0	23	2	-	-
	A	07	7	12	47.9	16	3	6	1
	A	09	26	4	18.2	19	8	-	-
	A	10	43	3	1.9	18	1	-	-
	A	12	42	2	7.7	6	5	-	-
						24	9	-	-
						17	5	-	-
						21	1	-	-
						12	1	-	-
	D	14 Lo	45	2	16.7	25	3	-	-
	D	20	6	13	79.4	11	2	-	-
	D	26 sh	2	27	77.6	9	5	3	4
	D	26 sh	13	8	90.3	10	7	-	-
	D	26 sh	14	6	50.6	31	-	-	-
						15	1	-	-

† Mapping population previously reported by Shappley et al. (1998).

‡ Mapping population previously reported by Ulloa and Meredith (2000).

and 20 QTLs for fiber quality traits) and spanned 117 cM marked with 54 RFLP loci. At least six linkage groups with putative QTLs from the two cotton populations coalesced into the largest joinmap linkage group, G-1 (Chr-03), explaining from 4.7%–38.5% of the trait variations (Table 2 and Figure 2). Linkage group 2 (long arm of Chr-26) harbored 19 QTLs: three QTLs for agronomic and 16 QTLs for fiber quality traits, covering 77.6 cM with 27 RFLP loci (Table 2 and Figure 4). Five linkage groups with detected QTLs blended into the second-largest linkage group, group 2 (long arm of Chr-26), explaining from 3.4%–44.6% of the trait variation. Ulloa and Meredith (2002) previously blended eight linkage groups (2, 4, 5, 9, 13, 16, 34, and 38) from the joinmap (Ulloa et al. 2002) and their associated QTLs (55) from the four populations (Pop 1, Pop 2, Pop 3, and Pop 4). At LOD 3.0, they observed a larger linkage group (130.5 cM) with more added loci and a reduced genetic distance between two markers, covering 130.5 cM with 93 RFLP loci. In this study, the joinmap linkage groups 2 and 13 and their associated QTLs (20) were shown to be part of the long arm of chromosome 26 and blended to the above previously reported (Ulloa and Meredith 2002), larger putative biological linkage group (Figure 4).

Assignment of the joinmap linkage groups to specific chromosomes and subgenomes (A versus D) of the *G. hirsutum* tetraploid cotton revealed that 63 QTLs were associated with five chromosomes of the A subgenome (Chr-03, Chr-07, Chr-09, Chr-10, and Chr-12) and 29 QTLs of the D subgenome from four chromosomes (Chr-14sh, Chr-20, the long arm of Chr-26) (Figures 2–4). This result agrees with previous studies that indicated genetic control of fiber trait variability in *G. hirsutum* may predominately occur on certain chromosomes (Shappley et al. 1998; Ulloa and Meredith 2000; Ulloa and Meredith 2002; Paterson et al. 2003). Some QTLs for yield component traits were located on these chromosomes.

The following traits—lp (LR=12.16), sdx (LR=12.3), e1 (LR=7.88–29.03), mic (LR=7.88–19.75), mat (LR=13.00–13.82), and per (LR=13.5)—were placed on linkage groups belonging to Chr-03 of the A subgenome. The following traits—sdx (LR=10.63), e1 (LR=10.75–17.58), 50% SL (LR=11.56), mat (LR=10.63), and wfns (LR=10.76)—were placed on linkage groups belonging to the long arm of Chr-26 of the D subgenome. Based on this study, the QTLs for various cotton traits tend to reside at or near the same locus for certain linkage groups from the two diverse populations (Table 2 and Figures 2–4).

Discussion

In this study, QTL examination on the joinmap for agronomic and fiber quality traits revealed highly recombined and gene abundant regions on cotton chromosomes. The maps presented herein were developed from intraspecific $F_{2:3}$ cotton populations whose parents arose from artificial selection and breeding at different locations, with differing germplasm pools, including Acala, Mid-south Upland, and Texas MAR germplasm. The compilation of genetic linkage maps and joinmaps from different breeding gene pools enabled partial dissection of the A and D subgenomes in *G. hirsutum*. Because RFLP markers were detected using cDNA probes, the results describe locations of expressed genes. Relative to other types of markers, the cDNA-derived RFLPs may be more likely than some other marker types to be physically and recombinationally close to the comprehensive array of economically important genes. Moreover, the data are relatively portable, so comparison is feasible with gene-rich regions of interspecific (*G. hirsutum*×*G. barbadense*) maps.

The increased marker density in the joinmap relative to the individual maps should facilitate both genetic and physical mapping of the cotton genome. It is evident that mapping at the multipopulation level has many advantages over that based on a single population. Gene order and map distances are estimated more accurately with a large number of mapped loci in different populations with different genetic backgrounds. Two major linkage groups of the joinmap, Group 1 (Chr-03) and Group 2 (long arm Chr-26), in two different chromosomes belong to A and D subgenome, respectively, indicate that cotton chromosomes may have regions of high (hot spot) and low (cold spot) meiotic recombination, like many other eukaryotic organisms (Gill et al. 1996; Petes 2001; see Figures 2 and 4). This presence of unevenly distributed, marker rich, and recombinationally active regions in two different A and D subgenome-specific chromsomes suggested that no correlation was present between the length of the chromosomes and the number of loci because normally A genome has larger-size chromosomes compared to the D genome chromosomes. However, the variation in marker density along linkage groups was primarly due to the presence of dense marker regions. Similar results were reported by Lacape et al. (2003), Paterson et al. (2003), Rong et al. (2004), and Mei et al. (2004).

Based on genome-specific chromosomes identified in *G. hirsutum* tetraploid (A and D), the A subgenome exhibited 68% of QTLs from the five chromosomes, while the D subgenome exhibited 32% of QTLs from the three chromosomes. The D subgenome, even though it does not produce spinnable fibers, has been found to possess QTLs positively affecting fiber and morphological traits (Reinisch et al. 1994; Wright et al. 1998; Jiang et al. 2000). In addition, other related studies revealed the uneven distribution in the tetraploid cotton (*G. hirsutum*) of A and D subgenomes as compared with their ancestral diploid genomes (Lacape et al. 2003; Paterson et al. 2003; Zhong et al. 2002; Rong et al. 2004; Mei et al. 2004). These maps presented herein were developed within the same species (*G. hirsutum*×*G. hirsutum*). The uneven distribution in the tetraploid cotton can be used as a basis for genotypic selection among individuals, accelerating introgression of desired chromosomal segments into a genetic background. In addition, based on this study, the QTLs for various cotton traits tend to reside at or near the same locus for certain linkage groups from the two diverse populations—for example, G-1 (Chr-03) and G-2 (long arm Chr-26)—which belong to A and D subgenomes, respectively (Table 2 and Figures 2–4).

The genetic linkage joinmap promises to provide a better understanding of cotton by possibly providing a core of markers with more practical application and/or by developing new markers with greater trait variation. In addition, QTLs for fiber quality traits on these linkage groups on certain regions are located between two RFLP markers on an average of less than one cM=0.4–0.6 megabases (Mb) and could be the target for map based cloning (physical mapping). Continuing research is being done for additional mapping of populations for QTL validation (from different gene pools and Recombined Inbred Lines) and for the identification of RFLP loci lineage for *G. hirsutum* from its diploid progenitors (the A and D genomes). Increased marker density in the joinmap would facilitate both genetic and physical mapping of the cotton genome.

Acknowledgments

The authors would like to thank Dr. Alex Kahler of Biogenetic Services, Inc., for the services provided in the RFLP assays. Names are necessary to report factually in available data; however, the USDA neither guarantees nor warrants the standard of products or services, and the use of the name by the USDA implies no approval of the product or service to the exclusion of others that may also be suitable.

References

Ausubel F (ed), 1978. Current protocols in molecular biology. New York: Green Publishing Company.

Austin DF and Lee M, 1996. Comparative mapping in $F_{2.3}$ and F6.7 generations of quantitative trait loci for grain yield and yield components in maize. Theor Appl Genet 92:817–826.

Basten CJ, Weir BS, and Zeng ZB, 1994. Zmap-a QTL cartographer. V. 22 In: Proceedings of the fifth world congress on genetics applied to livestock production: computing strategies and software, Guelph, Ontario, Canada, 7–12 August 1994. (Smith et al. eds.) Guelph, ON: University of Guelph; 65–66.

Churchill GA and Doerge RW, 1994. Empirical threshold values for quantitative traits mapping. Genetics 138:963–971.

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, and Specht JE, 1999. An integrated genetic linkage map of the soybean genome. Crop Sci 39:1464–1490.

Endrizzi JE and Ramsay G, 1979. Monosomes and telosomes for 18 of the 26 chromosomes of *Gossypium hirsutum*. Can. J Genet Cytol 21:531–536.

Endrizzi JE, Turcotte EL, and Kohel RJ, 1985. Genetics, cytology, and evolution of *Gossypium*. Adv Gen 23:271–375.

Gill KS, Gill BS, Endo TR, and Taylor T, 1996. Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144:1883–1891.

Jiang C, Wright RJ, Woo SS, DelMonte TA, and Paterson AH, 2000. QTL analysis of leaf morphology in tetraploid *Gossypium* (cotton). Theor Appl Genet 100:409–418.

Kianian SF and Quiros CF, 1992. Generation of *Brasica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theor Appl Genet 84:544–554.

Kohel RJ, 1973. Analysis of irradiation induced virescent mutants and the identification of a new virescent mutant (v5v5, v6v6) in *Gossypium hirsutum* L. Crop Sci 13:86–88.

Lacape J-M, Nguyen TB, Thibivilliers S, Bojinov B, Courtois B, Cantrell RG, Burr B, and Hau B, 2003. A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum*×*Gossypium barbadense* backcross population. Genome 46:612–626.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, and Newburg K, 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural population. Genomics 1:174–181.

Liu S, Cantrell RG, McCarty JC, and Stewart McD, 2000. Simple sequence repeat-based assessment of genetic diversity in cotton race stock accessions. Crop Sci 40:1459–1469.

Mei M, Syed NH, Gao W, Thaxton PM, Smith CW, Stelly DM, and Chen ZJ, 2004. Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*). Theor Appl Genet 108:280–291.

Paterson AH, Saranga Y, Menz M, Jiang C-X, and Wright RJ, 2003. QTL analysis of genotype × environment interactions affecting cotton fiber quality. Theor Appl Genet 106:384–396.

Percival AE, Wendel JF, and Stewart JMcD, 1999. Taxonomy and germplasm resources. In: Cotton (Smith CW and Cothren JT, eds). New York: J. Wiley and Sons; 33–63.

Petes TD, 2001. Meiotic recombination hot spots and cold spots. Nat Rev Genet 2:360–369.

Reinisch AJ, Dong JM, Brubaker CL, Wendel JF, and Paterson AH, 1994. A detailed RFLP map of cotton, *Gossypium hirsutum*×*Gossypium barbadense*: chromosome organization and evolution in a disomic polyploid genome. Genetics 138:829–847.

Rong J, Abbey C, Bowers JE, Brubaker CL, Chang C, Chee PW, Delmonte TA, Ding X, Garza JJ, Maler BS, Park Chan-C, Pierce JG, Rainey KM, Rastogi VK, Schulze SR, Trolinder NL, Wendel JF, Wilkins TA, William-Coplin TD, Wing RA, Wright RJ, Zhao X, Zhu L, and Paterson AH, 2004. A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (*Gossypium*). Genetics 166:389–417.

Saha S and Stelly DM, 1994. Chromosomal location of phosphoglucomutase 7 locus in *G. hirsutum*. J Hered 85:35–39.

Saha S, Jenkins JN, McCarty JC, and Stelly DM, 2000. Chromosomal location of RFLP markers linked to QTL in cotton. In: New Frontiers in Cotton Research: Proceedings of the World Cotton Research Conference, Athens, Greece, 6–12 September 1998. (Gillham FM, ed); Vol. 1: 204–205.

Saha S, Wu J, Jenkins JN, McCarty JC, Stelly DM, Percy RG, Raska DA, and Gutierrez AO, 2004. Effect of chromosome substitutions from *Gossypium barbadense* L. 3–79 into *G. hirsutum* L. TM-1 on agronomic and fiber traits. J Cott Sci 8:162–169.

Shappley ZW, Jenkins JN, Zhu J, and McCarty JC, 1998. Quantitative trait loci associated with agronomic and fiber traits of Upland Cotton. J Cott Sci 4:153–163.

Stam P and Van Ooijen JW, 1995. JOINMAP™ version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen.

Stelly DM, 1993. Interfacing cytogenetics with the cotton genome mapping effort. In: Proceedings of the beltwide cotton conference. New Orleans, LA, January 10–14, 1993. Memphis, TN: National Cotton Council; 1545–1550.

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, and Young ND, 1992. High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160.

Tomkins JP, Paterson DG, Yang TJ, Main D, Wilkins J, Paterson AH, and Wing RA, 2001. BAC library construction, preliminary STC analysis, and identification of clones associated with fiber development. Mol Breed 8:255–261.

Ulloa M, Cantrell RG, Percy R, Lu Z, and Zeiger E, 2000. QTL analysis of stomatal conductance and relationship to lint yield in an interspecific cotton. J Cott Sci 4:10–18.

Ulloa M and Meredith WR Jr, 2000. Genetic linkage map and QTL analysis of agronomic and fiber quality traits in an intraspecific population. J Cott Sci 4:161–170.

Ulloa M and Meredith WR Jr, 2002. QTL examination on a RFLP joinmap from four intraspecific cotton populations. In: Proceedings of the plant animal and microbe genome X conference. San Diego, CA, 12–16 January 2002. (Heller SR, et al., eds.) New York: Scherago International, Inc.; 247.

Ulloa M, Meredith WR Jr, Shappley ZW, and Kahler AL, 2002. RFLP genetic linkage maps from four F2.3 populations and a joinmap of *Gossypium hirsutum* L. Theor Appl Genet 104:200–208.

Van Ooijen WJ, and Maliepaard C, 1996. MapQTL^{tm} version 3.0: software for the calculation of QTL positions on genetic maps. Wageningen: CPRO-DLO.

Wendel JF, Burbaker CL, and Percival E, 1992. Genetic diversity in *Gossypium hirsutum* and the origin of Upland cotton. Am. J Bot 79:1291–1310.

Wright RJ, Thaxton PM, El-Zik KM, and Paterson AH, 1998. D-subgenome bias of *Xcm* resistance genes in tetraploid *Gossypium* (cotton) suggests that poplyploid formation has created novel avenues for evolution. Genetics 149:1987–1996.

Xiao J, Li J, Yuan L, and Tanksley SD, 1996. Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. Theor Appl Genet 92:230–244.

Yu J, Yong-Ha P, Lazo GR, and Kohel JR, 1998. Molecular mapping of the cotton genome: QTL analysis of fiber quality properties. In: Proceedings of the beltwide cotton conference. San Diego, CA, January 5–9, 1998. (Dugger P, and Richter D, eds.) Memphis, TN: National Cotton Council Of America; 485.

Zhong M, McCarty JC, Jenkins JN, and Saha S, 2002. Assessment of day-neutral backcross populations of cotton using AFLP markers. J Cott Sci 6:97–103.

Received March 24, 2004
Accepted August 15, 2004

Corresponding Editor: Reid Palmer